Arrays of 3D Double-Network Hydrogels for the High-Throughput Discovery of Materials with Enhanced Physical and Biological Properties

Supplementary Information

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Cairnan Duffy[1]‡, Andrea Venturato[1]‡, Anthony Callanan[2], Annamaria Lilienkampf[l], Mark Bradley[1,]*

[1]School of Chemistry, EaStCHEM, University of Edinburgh, Joseph Black Building, West Mains Road, Edinburgh, EH9 3FJ, UK. [2]Institute of Bioengineering, University of Edinburgh, Faraday Building, King's Buildings, Edinburgh, EH9 3JI, UK.

‡These authors contributed equally

*Corresponding author: mark.bradley@ed.ac.uk

Table S1. Procedures used to the production of the masks for array fabrication and mechanical testing.

Step	MoldStar Silicon – Array Mask	Sylgard 184 (PDMS) – Sample Mask
Mould Fabrication	Mould printed with Ultimaker 1(0.4 mm nozzle, 30 mm/s, 225 ºC).	Mould printed with Ultimaker 1 (0.4 mm nozzle, 30 mm/s, 225 ºC).
Mask Fabrication	1) The two commercial elastomer components are mixed in 1:1 ratio until homogeneous mixture is formed. 2) Mould is affixed to a glass slide and filled with the mixture and cured at room temperature for 6 h.	1) Sylgard 184 elastomer component and cross-linker are mixed in 10:1 ratio, stirred for 15 min, and degassed in vacuum oven for 15 min. 2) Mould is filled and cured overnight at 50 ºC.
Mask Removal	The mould is removed and the silicon-based mask is peeled-off from the glass slide. Silicon layer present on each reaction well is gently removed.	Mask is extracted from the mould.
Mask Use	Mask placed on an agarose-coated glass slide. Hydrogel components are dispensed inside each reaction well using a bioprinter and polymerised *in situ*.	Components of the hydrogels are added to the mask "wells" (cylindrical and dog-bone shapes) and polymerised *in situ* to allow the formation of hydrogels with specific dimensions.

Table S2. Monomer combinations for the fabrication of the first network hydrogels at 8, 12, 16, and 20 mol% MBA cross-linker concentration.

Monomer 1	Monomer 2	Monomer Ratio	
AEtMA-Cl	NIPA	5	3
AEtMA-Cl	DMOBAA	5	3
DMAA	DMAEMA	3	1
AEtMA-Cl	DEAA	1	1
AEtMA-Cl	NIPA	1	3
AEtMA-Cl	NIPA	3	1
AEtMA-Cl	DEAA	3	1
AEtMA-Cl	DMOBAA	1	1
CEA	DMAEMA	2	5
AEtMA-Cl	-	-	-
DMOBAA	-	-	-
AEtMA-Cl	DMAEMA	1	1
EMAP	DMAEMA	1	3
AEtMA-Cl	DEAA	1	3
CEA	-	-	-
NIPA	-	-	-
DMOBAA	NIPA	7	1
PEG_6MA	DMAEMA	3	1
DEAA	NIPA	1	1
DMOBAA	NIPA	1	7

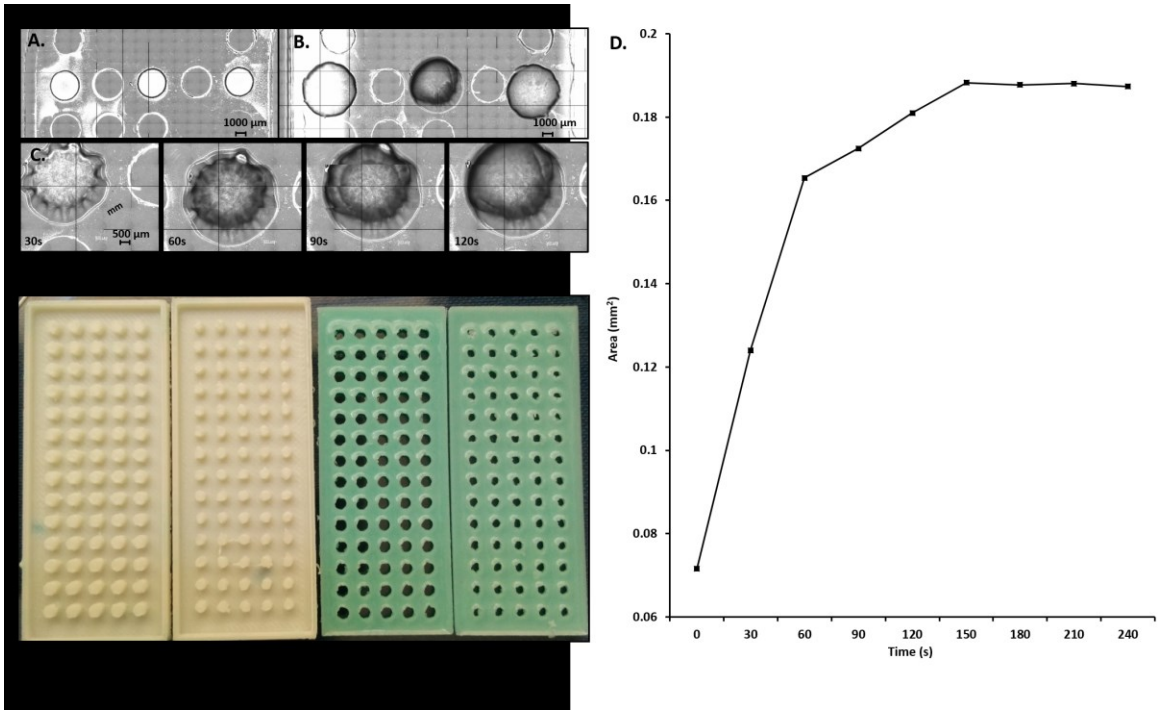

Figure S1. (A–D) Examination of the swelling ratio of a representative hydrogel AEtMA-Cl:DEAA (1:1) with 12 mol% MBA. This was used to determine the optimal distances between the features on the arrays. (A) Triplicate hydrogel features (4 µL) prior to hydration on a glass slide. (B) Triplicate hydrogel features hydrated after 240 seconds of submersion in water. (C) Hydration/swelling of a 4 µL hydrogel feature after 30, 60, 90 and 120 seconds. (D) Swelling (mm^2) of a hydrogel feature on a glass slide over 240 seconds. (E) Poly lactic acid (PLA) moulds of different dimensions of cylindrical features (white) used to create the silicon elastomer masks with 'reaction wells' (green).

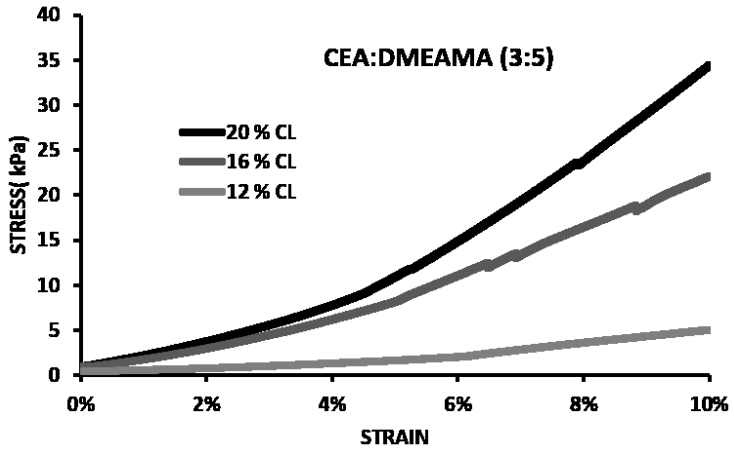

Figure S2. Representative stress/strain curves for the double-network hydrogel CEA:DMEAMA (3:5 ratio, respectively) with a first network concentration of 1 M at 12, 16 and 20 mol% MBA and a second network poly-acrylamide concentration of 2 M with 0.1 mol% MBA. Compression stiffness modulus was at a compression rate of 0.5 %/min until a strain of 10 % of the sample hight was reached.

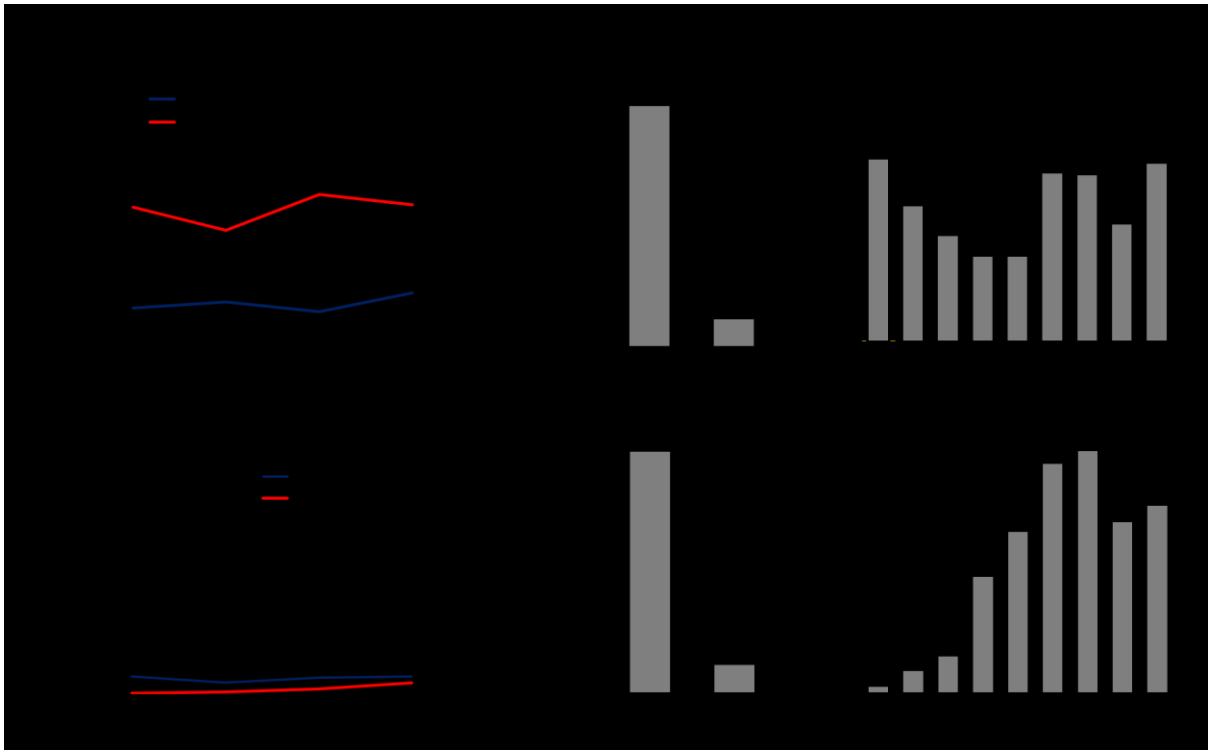

Figure S3. The effect of the formulation of double-network (polyacrylamide) AEtMA-Cl:DEAA on tensile strain failure (%) (top) and compressive modulus (kPa) (bottom). (A) The effect of the concentration of the first network monomers on compressive modulus with different concentrations of acrylamide to form the second network (n = 4). (B) The effect on compressive modulus of different cross-linker concentrations used to form the second network (n = 4). (C) The effect on compressive modulus of changing the monomer ratios of AEtMA-Cl:DEAA (at 16% cross-linker concentration) from the homo-polymer of AEtMA-Cl (designated A) to the homo-polymer of DEAA (designated D) (n = 3). (D) The effect of the concentration of the first network monomers on tensile failure strain with different concentrations of acrylamide to form the second network (n = 4). (E) The effect on tensile strain failure of different cross-linker concentrations used to form the second network. (F) The effect on tensile failure strain of changing the monomer ratios of the AEtMA-Cl:DEAA hydrogel (at 16% cross-linker concentration) from the homo-polymer of AEtMA-Cl (A) to the homo-polymer of DEAA (D) (n = 3).